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THE INFLUENCE OF CREOSOTE, GUAIACOL AND RELATED SUBSTANCES ON THE TUBERCLE BACILLUS AND ON EXPERIMENTAL TUBERCULOSIS

STUDIES ON THE BIOCHEMISTRY AND CHEMOTHERAPY OF TUBERCULOSIS. XIX.

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Of the countless drugs used in tuberculosis, creosote and its derivatives undoubtedly hold first place in extent of use and general reputation, but absolutely without scientifically established value. Perhaps as good a summarization of their status as any is given by Lawrason Brown,[1] who says that creosote and its derivatives "are the most used of false specifics. They have never been proved to exert any action on the tuberculous process but in some patients have almost a specific action upon the accompanying secondary infection of the lungs, such as simple bronchitis. They also exert a very stimulating effect upon the bronchial mucous membrane during their excretion through it." Other authors vary somewhat in their judgment, but positive statements are avoided, of necessity. Kobert says, "Very little that is conclusive can be said concerning the usefulness of these preparations." Bandelier Ropke's "Die Klinik der Tuberkulose" contains the conclusion that "Creosote and guaiacol preparations are not internal disinfectants, but for certain cases they stimulate the appetite and improve digestion. Their routine use is therefore by no means justifiable."

A careful examination of the extensive literature on the many compounds of this class indicates that opinions as to their value or action, whether favorable or unfavorable, rest upon very slender evidence. General clinical experience is the only extensive source of information, and this is, of course, uncontrolled and conflicting, so it

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[1] Klebs: Tuberculosis, New York, D. Appleton & Co., 1909.

does not serve as a basis for conclusions of any value whatever. The most that can be said is that the use of creosote therapy in tuberculosis has persisted so long and so widely that it seems probable that some beneficial results must have been observed. Medical history gives us the right to suspect that any methods or substances of therapeutics that continue to be generally used for many years have some definite favorable effect, although the real establishment of their principles and limitations may not be understood. Sometimes after years of clinical use, and sometimes even after long subsequent years of disuse, the explanation of their activity has been established.

There has been no lack of endeavor by chemical manufacturers to provide all possible derivatives of creosote and its components, as can be seen in any list of chemical products, but especially in Fraenkel's "Arzneimittelsynthese." These endeavors have, with few exceptions, not been based on considerations of bactericidal efficiency, and indeed this essential aspect has been almost completely neglected. As Fraenkel says in summarizing this section of his book, two objects underlie the preparation of these multitudinous derivatives of creosote and guaiacol—(a) reduction of the toxicity and irritating properties, together with improvement in taste; (b) water solubility. Most of the products accomplishing the first objects, largely by means of the salol principle, are not water-soluble. The water-soluble compounds are mostly bad tasting, and, in case of the sulfonates, are much reduced in activity. "In therapeutic application, in the first rank of products indisputably come those formed by esterification of the hydroxyl group." He also comments that of the active components of creosote only the guaiacol has had general use in pure form, while the less toxic cresols, which have analogous effects, have had no consideration.

There are numerous compilations of literature on creosote therapy in tuberculosis (under which term are included the numerous derivatives of creosote and related substances), and we shall not give another compilation. One of the best reviews is that by von Weismayr in Ott's "Chemische Pathologie der Tuberculose." We give, however, in brief form such scanty evidence as we have found concerning the bactericidal action of this class of compounds, both in vitro and in vivo:

Bouchard is quoted by Weismayr as having found that 0.8 per 1,000 strength of creosote in glycerol bouillon retards the growth of tubercle bacilli, and 0.5 per 1,000 is effective in blood serum. In daily doses of 0.25 gm. per kilo it caused immunity to tuberculosis in rabbits, so that animals killed after three months showed no lesions. We regret that we have not been able to find the origin of this statement, for it is perhaps the most definite one in the literature.

On the other hand, Cornet[2] reported that he inoculated 7 guinea-pigs with tuberculosis after they had been given 0.02 gm. creosote daily per catheter into the stomach for about a month, and continued after infection, but they all died of tuberculosis as did the control animals. This dose he estimated as corresponding to 2.0 gm. creosote per day in a man. He also quotes Schüller as obtaining positive results and Sormani and Pallacani as obtaining negative results by causing infected guinea-pigs to inhale creosote.

Guttmann[3] studied the inhibition of growth of different species of bacteria on gelatin containing creosote, which he found to be more effective than phenol. Of 18 species of bacteria, 12 failed to grow when the gelatin contained one part in 2,000 (including 4 not growing at 1:4,000), and 5 of the remaining 6 failed to grow when the concentration was 1:1,000. With 17 species on gelatin containing phenol, 12 grew when the concentration was 1:2,000. Tubercle bacilli were partly inhibited by creosote 1:4,000 and even 1:16,000, but it required 1:2,000 to prevent growth entirely. He then speculated as to the possibility of obtaining a concentration of 1:2,000 of creosote in the human body, and found that this could not be done, wherefore he was doubtful as to the efficiency of creosote in tuberculosis—a speculation that has been quoted extensively in literature on tuberculosis.

Fraenkel[4] corroborated in 1889 the statement that cresols are stronger antiseptics than phenol. He quotes von Behring's estimate that one-sixth the amount of an antiseptic found to be inhibiting for bacteria represents approximately the lethal dose for an animal. In accordance therewith, Fraenkel found that 1:300 cresol sulfate is inhibiting to bacteria (species not stated) which would correspond on this basis to two-sixths gm. in a 600 gm. pig, an amount found fatal although a slightly smaller dose was not fatal.

Kuprianow[5] quotes Petrescu as finding that tubercle bacilli did not grow in 10 c c bouillon containing 4.1 gm. (sic) guaiacol, and that tubercle bacilli exposed to guaiacol did not produce tuberculosis. Marfori also found that guaiacol rendered tubercle bacilli unable to infect rabbits. No other references to direct observations on the bactericidal power of guaiacol could be found in the literature to 1894 by Kuprianow. He therefore made some tests by Loeffler's method, in which the solution to be tested is poured over the surface of an inoculated agar slant, and poured off again after a specified time, the tube then being incubated. In other experiments 24 to 48 hour slant cultures were exposed to the solutions and material removed with a platinum loop was inoculated on fresh mediums. The inhibiting effect in bouillon cultures was also determined. Phenol and creosote were found by these methods to be about equally destructive to staphylococci and B. pyocyaneus, but guaiacol was less active than either. To all 3 the staphylococcus was much more resistant than the pyocyaneus bacilli. Tubercle bacilli were exposed in masses of culture to 4% alcoholic solution of guaiacol and creosote for 15 seconds to 2 hours, and after pouring off the solution no growth followed. Obviously these last experiments are too crude to be of any significance.

Koch is quoted as having found that creosote inhibits the growth of tubercle bacilli in cultures, but the reference cited in the literature is incorrect, and the original article has not been located.

[2] Ztschr. f. Hyg. u. Infektionskr., 1888, 5, p. 124.
[3] Ztschr. klin. Med. 1888, 13, p. 488.
[4] Ztschr. f. Hyg. u. Infektionskr., 1889, 6, p. 521.
[5] Centralbl. f. Bakteriol., 1894, 15, p. 933.

Winkler[6] exposed agar plate cultures of tubercle bacilli to vapor from a mixture of guaiacol and iodoform for eight days and found that the material became noninfectious. Injection of this mixture into animals did not save them from tuberculosis, and serum of rabbits injected with the antiseptic had no effect on infection with tubercle bacilli.

Villa is quoted by von Weismayr as having found that guaiacol prevents growth of streptococci in a dilution of 1:1,000, and kills in this dilution in 16 minutes, and in dilution of 1:100 in 2 minutes.

Hammerl[7] found that paracresol is equal to orthocresol in bactericidal power against staphylococci and typhoid bacilli, but more toxic. Phenol was less strongly bactericidal and more toxic than either.

Several authors quote Shaw[8] as having demonstrated that guaiacol is ineffective in infections of animals, but the original article shows that he merely inoculated two rabbits with B. pyocyaneus, and injected one with 20 c c of a 1:200 guaiacol solution (the lethal dose of which is 25 c c). This animal died in 18 hours and the control in 26 hours. There is no other experimental evidence in this much quoted article.

One of the most important contributions to the subject of creosote therapy is that of Bechhold and Ehrlich,[9] who (using especially diphtheria bacilli for their tests) developed many new and fundamental facts in relation to the influence of various modifications of the phenol derivatives on their bactericidal and physiologic action. The chief conclusions were:

1. Introduction of halogens into phenol increases the disinfectant action in proportion to the number of halogen atoms introduced[10] (e. g., 1 mol. pentabrom phenol has the same action on diphtheria bacilli as 500 mol. phenol).

2. Alkyl groups introduced into phenols or halogen phenols increases their disinfectant action. (Tribrom-m-xylenol is 20 times as active as tribrom phenol; tetrabrom-o-cresol is 16 times as active as tetrachlor phenol).

3. Union of 2 phenols or halogen phenols, either directly or through CH_2, CHOH, $CHOCH_3$ or $CHOC_2H_5$ groups, increases activity. Thus, tetrabrom-o-cresol inhibits the growth of diphtheria bacilli in a dilution of 1:200,000, while tetrabrom-o-biphenol inhibits when dluted to 1:640,000.

4. Union of 2 phenols through CO or SO_2 decreases activity.

5. Introduction of COOH into the nucleus decreases activity.

6. Halogens introduced into phenols at first reduce toxicity, but the trihalogens have about the same toxicity as the unhalogenized substance, and tetra- and penta-halogen compounds are more toxic. However, the spasmodic action of the phenols is reduced in proportion to the number of halogen atoms. CH_3 groups compensate or neutralize the toxicity introduced by the halogens.

Of the compounds developed in this study the most effective were:

Tetrabrom-o-cresol, which has but little toxicity yet inhibits growth of diphtheria bacilli diluted to 1:200,000, and in 1% solution kills them in less than 2 minutes. It compares in activity with phenol in the ratio of 1,000:0.9.

Tetrabrom-o-biphenol (and the corresponding Cl compounds) which is more toxic but inhibits growth at a dilution of 1:640,000.

Hexabrom-diphenyl carbinol, practically nontoxic, inhibits growth at 1:200,000; kills in 24 hours in dilution of 1:320,000 and kills in 10-15 minutes at

[6] Deut. med. Wchnschr., 1893, 19, p. 781.

[7] Hyg. Rundschau, 1899, 9, p. 1017.

[8] Jour. of Hygiene, 1903, 3, p. 159.

[9] Ztschr. physiol. Chem., 1906, 47, p. 173.

[10] In a Patentschrift, Dammann, in 1889, also mentions this effect of halogens· quoted by Schottelius, Arch. f. Hyg. 1913, 82, p. 76.

1:1,000. Compares with phenol as 1,000 to 0.6 in respect to action on diphtheria bacilli, although less effective against "water bacteria" than phenol.

Although these substances did not precipitate proteins they were ineffective against diphtheria bacilli in serum, and on this basis the authors explain their failure to influence favorably diphtheria infection in animals Unfortunately, they give no details as to the methods used in conducting these experiments. They merely say "Wir versuchten Hexabromdioxydiphenyl carbinol, Tetrabromhydrochinonphthalein usw. besonders gegen Diphtherie an Meerschweinchen, Kaninchen, und auch gegen Streptococcen an weissen Mäusen, Tetrabrom-o-Kresol gegen Streptococcen an weissen Mäusen. Der Erfolg blieb aus."

This article was followed by a report by Bechhold[11] under the title of "Halbspezifische chemische Desinfektionsmittel" in which is emphasized the fact that the effect of a given chemical on one species of bacteria may not be duplicated with another species, and hence general laws covering the influence of various modifications of a substance on its bactericidal action cannot be deduced from limited observations. Thus, in the previous article it was stated that the introduction of halogen atoms into phenols increases the disinfectant action somewhat in proportion to the number of added halogens. But Bechhold finds that against staphylococci, streptococci and diphtheria bacilli the maximum disinfectant power is shown by tribrom-β-naphthol, as compared with either mono- and di- or tetra- and penta-brom-β-naphthol. On the other hand, against paratyphoid bacilli the activity is constant as halogens are added to dibrom or dichlor, and decreases with three or more halogen atoms. The "semi-specificity" of this class of disinfectants is shown by several examples. Thus, tetrabrom-p-biphenol and tribrombikresol are very actice disinfectants for staphylococci, but against colon bacilli they are less effective than lysol. While tri- or tetrabrom-β-naphthol, tetrabrom-o-cresol and tetrachlor-l-biphenol have a considerable disinfectant action even on anthrax spores, they as well as some others of the higher halogen phenols, are practically inactive against tubercle bacilli. Tetrabrom-o-cresol, hexabromdioxydiphenyl-carbinol, tetrachlor-o-biphenol, tetrabrom-biphenol and tribromcresol, in 1% solution for 2 hours with an emulsion of human tubercle bacilli did not impair their infectivity for animals. Tri- tetrabrom-β-naphthol acted in 2.5% solution on tubercle bacilli for 25 hours without effect, while a 5% lysol solution (containing 2.5% of cresol) kills tubercle bacilli in 4½-8 hours. Hence all these disinfectants which are much more actively destructive of staphylococci than lysol, are much less effective than lysol against tubercle bacilli.

As far as we can learn, the leads given in these articles have not been followed much farther, either in Ehrlich's laboratory or elsewhere. Leubenheimer[12] has established anew the general applicability of the principle of the effect of halogenized phenols to bacteria, and also demonstrated for different xylenes a high bactericidal action (Schottelius).

Raschig[13] is said to have followed this lead and produced a chlorinated xylenol which has great bactericidal properties.

Schottelius[14] has investigated the action of "grotan," described as "a complex cresol alkali compound," Na-p-chlor-m-cresol. This substance he found to be strongly bactericidal, 0.5% solution killing in 5 minutes all the bacteria

[11] Ztschr. f. Hygiene u. Infektionskr., 1909, 64, p. 113.

[12] Phenol und seine Derivate, Berlin, 1909; quoted by Schottelius.

[13] An incorrect reference to Raschig's work is given and we have not succeeded in locating the correct reference.

[14] München. med. Wchnschr., 1912, 59, p. 2674.

tried (typhoid stools and cultures, staphylococci, streptococci and pus), 0.3% usually being effective, and 0.25% killing in 20-30 minutes; 1% solution killed anthrax spores in 20 minutes. Tuberculous sputum was treated with an equal volume of 2% grotan for 10, 30 and 60 minutes and each injected into guinea-pigs and rabbits without infection resulting, although all the controls were tuberculous after 28 days. No further or more exact tests seem to have been made with tubercle bacilli. The substance is said to be almost nontoxic and nonirritating, 3 gm. subcutaneously not poisoning 4,000 to 6,000 gm. dogs.

He also reports[15] that in a mixture of chlorxylenol and chlorcresol the disinfectant action is not merely the sum of the components, but is increased about 100% above this. Espécially efficient is a preparation made by dissolving chlorxylenol in soap and adding to "grotan." A new compound called "sagrotan" is produced in this way, but no exact statement is made as to its preparation or composition, beyond announcing its production by Schülke and Mayr. This substance he found much more strongly bactericidal for anthrax spores, staphylococci, streptococci, typhoid bacilli, and pus or dejecta containing these organisms, than either lysol or liquor cresoli saponatus. Also tuberculous sputum was disinfected by 2% sagrotan in 2 hours, as shown by animal inoculation. It is almost entirely devoid of either local or systemic toxicity. Dogs took 10 gm. per kilo (1% of body weight) into the stomach without serious effects, and Schottelius took 15 gm. at one dose and held his arm 40 minutes in a 10% solution of sagrotan without serious effects. However, 100 gm. in 3 days to a 7.5 kilo dog did not remove intestinal bacteria.

Sagrotan was found by Friedenthal[16] to have an alkalinity, as sold in 10% solution, corresponding to 0.56% KOH, so that injected subcutaneously it causes local necrosis. Guinea-pigs are not affected by subcutaneous injection of 2.5 gm. per kilo. In general, the low toxicity of grotan and sagrotan was corroborated by Friedenthal, who does not discuss their bactericidal effects.

Fehrs,[17] in discussing various preparations of liquor cresoli saponatus, mentions that Staph. pyogenes aureus is very susceptible, and Streptococcus pyogenes intermediate.

An extensive consideration of the effect of electrolytes in disinfection with cresol soaps is given by Frei,[18] but this contains nothing bearing directly on our problems.

Heukeshoven[19] has made the most extensive study of the action of thiocol, and one of the few studies of the effect of creosote derivatives on tuberculous animals that we can find in the literature. He found that a-thiocol,  and b-thiocol,  were almost absolutely without inhibiting effect on staphylococci, anthrax and B. pyocyaneus, for all these grew in asparagin glucose agar containing from 1 to 5% of these compounds; while guaiacol carbonate, being used merely as a suspension, had little more effect, but K-guaiacolate prevented growth of staphylococci in 0.5% (the lowest concentration tried), and inhibited anthrax and pyocyaneus at 2% but not at 1%.

The animal experiments were performed with four series of rabbits inoculated with tubercle bacilli (origin not stated) in the eye. In each series were

[15] Arch. f. Hyg., 1914, 82, p. 76.

[16] Berl. kl. Wchnschr., 1915, 39, p. 1019.

[17] Centralbl. f. Bakteriol., 1. 1904, 37, p. 730.

[18] Ztschr. Infektkr. d. Haustiere, 1914, 15, p. 273 and 407.

[19] Experimentelles über die Wirkung des "Thiocols" bei der Tuberkulose, R. Heukeshoven, Inaug. Dissert., Bern, 1899.

nine rabbits, one serving as a control. They received daily doses of 0.5 gm. of each of the 4 guaiacol derivatives mentioned above, by means of a catheter. In one series the drug was given for 14 days before infection and not afterward; in the second the treatment was continued; in the third there was no preliminary treatment, but treatment continued after the infection; in the fourth treatment was not begun until 4 weeks after the inoculation. The result was, in brief, that in the rabbits receiving thiocol-a, 2 recovered completely and in none was the disease disseminated through the body; with thiocol-b, none recovered, in 6 there was no dissemination, and in 2 there was dissemination; with potassium guaiacolate, in 5 of 8 the tuberculosis was disseminated; with guaiacol carbonate it was disseminated in 6 of 8, and in the 4 controls. He also found increase of weight in animals given thiocol-a or guaiacol carbonate, potassium guaiacolate had a deleterious effect on the animals and thiocol-b had no effect. As it has been found that thiocol is excreted unchanged, and as it seems to be devoid of bactericidal properties (although not tested on tubercle bacilli) these favorable effects are difficult to explain.[20]

Lubowski[21] has reviewed the abundant literature on thiocol to the middle of 1904, but this literature contains no work that seems to be accurate except that of Heukeskoven, nor have we found any contributions of importance since that date.

Diethylglycine-guaiacol, $C_6H_4(OCH_3)(O\text{-}CO\text{-}CH_2\text{-}N\text{-}(C_2H_5)_2\ HCl)$

known commercially under the name Guiasanol, has been produced and described by Einhorn[22] as having many therapeutic advantages. Gastric irritation is avoided because the free base is not formed until the HCl is split off in the intestinal alkalies, and free guaiacol is eventually liberated since it is found in the urine.[23] The toxicity is low, rabbits being uninjured by 2 gm. subcutaneously, or 4 gm. by stomach, and 2% solutions are not irritating. Buchner tested the bactericidal action of guiasanol, finding it low in vitro, for it inhibited the growth of B. coli, B. pyocyaneus, B. proteus and S. pyogenes aureus only in concentrations of 1:50 or 1:100. If it is correct that guaisanol readily liberates guaicol in the body, the low bactericidal power in vitro is of little significance, and Einhorn claims that it is efficient as a local antiseptic in ulcerating cancer and tuberculous enteritis.

A few other incidental references complete the list.

Burow[24] reported that a 3% aqueous solution of sodium or potassium guaiacolate containing 0.01% potassium arsenite prevented growth of tubercle bacilli, although without the arsenic the guaiacol was ineffective. He also stated that the blood of a rabbit treated with this mixture would not support growth of tubercle bacilli, and that guinea-pigs and rabbits thus treated resisted tuberculous infection. These results he attributed chiefly to the arsenic. This

[20] Lynosol, which differs from Thiocol in having Ca in place of K, is said by Takenaka (Japanese Jour. Tuberc. Kekkakau Zassi, 1919, 2, No. 1) to be as strongly bactericidal for tubercle bacilli as creosote and guaiacol, which all, he says, inhibit growth at concentrations over 1:10,000.

[21] Allg. Med. Centr.-Ztg., 1905, 74, pp. 337, 356, 396.

[22] München. med. Wchnschr., 1900, 47, p. 10.

[23] Concerning absorption and elimination of guaiacol see Eschele, Ztschr. klin. Med., 1896, 29, p. 197.

[24] München. med. Wchnschr., 1910, 57, p. 1792.

[25] München. med. Wchnschr., 1911, 58, p. 2669.

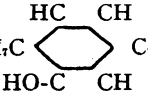
work, the report of which does not give a convincing impression, was repeated by Nürnberger,[25] who found no effects produced in either cultures or animals by the guaiacol-arsenic mixture of Burow, or by 0.01 gm. Na guaiacolate in glycerin-agar, the concentration not being stated.

J. Naberly[26] reported favorable clinical effects with a "new guaiacol chlor-iodid compound," the exact nature of which is not given. As there is no experimental evidence in regard to this compound, it is mentioned only because it has some possible relation to Ehrlich's observations. The Lancet laboratory examined this compound and found the iodin and chlorin nearly all in chemical union.

Cooper[27] reports an extensive study of creosote and allied substances, particularly with reference to the disinfectant action of various soap solutions in surgery and disinfection work. The chief facts developed of interest in this connection are:

Using the Rideal-Walker method as modified by Chick and Martin, the phenol coefficient of the cresols in pure aqueous solution was found to be:

	B. typhosus	S. pyogenes aureus
Ortho cresol	2.6	2.1
Meta cresol	2.6	2.0
Para cresol	2.6	2.4
Thymol	25.0	...
"Cresylic acid"	...	2.2

Therefore, as shown by thymol, C_3H_7C  $C\text{-}CH_3$, alkyl groups in the benzene ring may increase greatly the germicidal action, which accords with Bechhold's and Ehrlich's statements, and with the observation made by Koch in 1881.

On the other hand, the introduction of a second OH group into phenol decreases bactericidal action. Thus, for typhoid bacilli in water the phenol coefficient of various OH compounds was: resorcin, 0.3; pyrocatechin, 0.5; hydroquinone, 1.0; pyrogallol, 0.77; phloroglucin, less than 0.35. Quinone, O  O, however, had a phenol coefficient for staphylococci of 10, whereas acetone was less than 0.075.

Krauss,[28] in describing several new compounds of trypan red, gives the method of making guaiacol trypan red and iodo-guaiacol trypan red. He says nothing concerning their activity, but Paul Lewis in a brief note published elsewhere[29] states that no effects had been obtained in experimental tuberculosis with any of their trypan red compounds.

BACTERICIDAL AND BACTERIOSTATIC EXPERIMENTS

In order to determine whether there is any reason for believing that the various members of the guaiacol series should be expected to have any direct action on tuberculosis, the inhibitory or bacteriostatic action

[26] Lancet, 1913, 2, p. 285.
[27] Brit. Med. Jour., 1912, 1, p. 1234.
[28] Jour. Amer. Chem. Soc., 1914, 36, p. 960.
[29] Jour. Pharm. and Exper. Therap., 1914, 4, p. 353.

of a considerable number of the series has been tested on the bacillus of human tuberculosis. Several strains of the organism were used; in some cases no note as to the strain was made. In most of the tests, a strain which had been growing in our laboratory for a number of years and which we have distinguished from other strains acquired more recently, by the name "old human" was used. This strain has diminished somewhat in virulence but has not changed its growth characteristics. We use the following method for testing inhibitory power: To tubes containing a certain definite amount of glycerol agar, the chemical to be tested is added in sufficient quantity to make the desired dilutions. The dilutions range from 10% up to 0.0001%. Two of the substances used (styracol and guaiacol cacodylate) are quite insoluble in water. To make dilutions of these, the required amount of dry powder was added to hot agar, well shaken, and the agar cooled and slanted quickly before the powder settled out. Table 1 gives a summary of the experiments on the inhibitory action of all the tested drugs of this series.[30]

From this table, it may be seen that 0.01% or 1 in 10,000 dilution completely inhibited the growth of the tubercle bacillus in the test with resorcin, thymol, p-cresol, m-cresol and o-cresol; that 0.05% was the lowest concentration which completely inhibited in the case of creosol and pyrocatechin; guaiacol, creosote. hydroquinone and guaiacol cacodylate required a concentration of 0.1% or 1 in 1,000 to inhibit growth completely. Sodium guaiacolate inhibited completely at 1.7% and partially at 0.8%. Thiocol and styracol caused no inhibition at 1% concentration while the organisms grew well even in a suspension of 10% of styracol, which seems to be almost completely insoluble in water.

The bactericidal power of many of these compounds was then tested in the following way. The "old human" strain was used in all the tests. Six dilutions of the chemicals to be tested, from 1 in 100 to 1 in 1,000,000, were made in water. Small clumps of cultures were then immersed in these solutions, remaining in them 10 minutes, 1 hour, 6 hours, 24 hours and 48 hours. At the end of the desired time, the clump was removed, washed in several waters to remove the chemical and finally planted in agar tubes. Controls were made by exposing the clumps to normal salt solution for the same periods of time and then washing them in the same way. They all grew luxuriantly.

[30] Some of these experiments were carried out by Dr. Rachel Donnell.

The tubes inoculated with the clumps which had been exposed to 1 per cent. orthocresol for one hour developed a slight growth, while the 6 hour, 24 and 48 hour sets showed no growth. One per cent. metacresol, paracresol and thymol killed all the cultures after one hour's exposure, while 1 per cent. thymol killed all the organisms in 10 minutes. There was but little growth in the tubes inoculated with the clumps exposed for 10 minutes to 1 per cent. metacresol and paracresol. Creosol, resorcin, hydroquinone and pyrocatechin had no bactericidal effect in this experiment even in 1 per cent. concentration for 48 hours

TABLE 1

Inhibition of Growth of Human Tubercle Bacilli

Per-centage	Creosote			Guaiacol			O-cresol			M-cresol			P-cresol			Thymol			Creosol			Resorcin		
1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.8																								
0.5										—	—	—	—	—	—				—	—	—			
0.1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
0.05							—	—	—	—	—	—	—	—	—	—	—	—	—	—	—			
0.01	+	+	+	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	++	++	++	—	—	—
0.001	++	++	++	++	++	++	+	+	+	+	+	+	+	+	+	+	+	+	+++	+++	+++	+	+	+
0.0001	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	+++	+++	+++	+	+	—

Blank spaces indicate that that dilution was not used.
The — sign shows that there was no growth (complete inhibition).

and concentrations below 1 per cent. had no bactericidal effect with any of the drugs, even 0.1 per cent. thymol failing to kill in 48 hours, although 1 per cent. killed completely in 10 minutes.

Creosote and guaiacol were also tested by the same method but different dilutions were used. One hundred per cent. of either creosote or guaiacol killed all the organisms in 5 minutes so that there was no growth on the agar tubes. Five per cent. dilution killed some in 5 minutes and killed all in 1 hour; 1% creosote killed all in 1 hour and in 6 hours, but it required 6 hours for complete tuberculocidal action of 1% dilution of guaiacol. There was no growth in tubes after one hour's exposure to 0.5% dilution of creosote and 6 hours and 24 hours exposure to 0.1% creosote killed some of the organisms. Guaiacol, on the other hand, showed little bactericidal action in concentrations lower than 1%.

From these results, it may be seen that the bactericidal power of these substances is much lower than the bacteriostatic power. One per cent. was the only concentration which had any marked bactericidal power as shown by this method; 1% thymol killed all the organisms even in 10 minutes, so that they failed to grow when planted on agar tubes; 1% of metacresol and paracresol killed in 1 hour and of orthocresol in 6 hours. Even 1% of creosol, resorcin, hydroquinone or pyrocatechin failed to kill. One per cent. creosote killed K_4 bacillus in one hour and the Y. Miller strain was killed in one hour by 0.5% of creosote. It required 5% guaiacol to kill the "old human" in 1 hour although 1% killed all the organisms in 6 hours.

TABLE 1—*Continued*

INHIBITION OF GROWTH OF HUMAN TUBERCLE BACILLI

Thiocol			Styracol			Sodium Guaiacolate			Guaiacol Cacodylate			Hydro-quinone			Pyro-catechin		
+++	+++	+++	+++	+++	+++				−	−	−	−	−	−	−	−	−
						− +	− +	− +									
+++	+++	+++	+++	+++	+++				−	−	−	−	−	−	−	−	−
+++	+++	+++				+	+	+	−	−	−	−	−	−	−	−	−
+++	+++	+++	+++	+++	+++				+	+	−	++	++	++	−	−	−
+++	+++	+++	+++	+++	+++	+	+	+	+++	+++	+++	+++	+++	+++	+	+	+
+++	+++	+++	+++	+++	+++	+	+	+	+++	+++	+++	+++	+++	+++	+++	+++	+++
+++	+++	+++	+++	+++	+++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++

The + sign shows growth, ++ more luxuriant growth and +++ very luxuriant growth. Controls were grown in all cases and always showed very luxuriant growth.

We realized that the clump and test tube method of bactericidal work could not be considered absolutely reliable, since some of the clumps may not be completely permeated by the chemical while others will be saturated and broken up so that they are difficult to transplant to new mediums. For these and other reasons, the results as given above are not entirely consistent. Therefore it seemed best to repeat the bactericidal experiments with some, at least, of the compounds, using more delicate methods for determining the results. We chose the so-called garnet method, which we used thus:

1. A sufficient number of garnets of fairly uniform size to allow ten for each animal, were thoroughly cleaned with sulphuric acid, water, acetic acid, water, alcohol and finally ether. They were well

dried and sterilized. A suspension of tubercle bacilli was then made in which the garnets were well shaken and allowed to stand for a time. The fluid was decanted off and the garnets dried over sterile calcium chlorid. When dry, the required number of garnets were placed on perforated platinum baskets and immersed in about 50 c c of the various dilutions for the desired periods of time. The dilutions used were 1%, 0.5%, 0.1% and 0.01% and the periods of exposure were 20 minutes, 1 hour, 6 hours and 24 hours. Controls were exposed to physiological sodium chlorid solution for the same periods of

TABLE 2

Animal Test of Bactericidal Action

Dilutions	Duration of Exposure	Creosote						Guaiacol					
		I		II		III		I		II		III	
		Local	General	Local	General	Local	General	Local	General	Local	General	Local	General
1%	20 minutes	+	—	—	—	+	—	—	—	+	—	+	—
	1 hour	—	—	—	—	—	—	+	—	+	—	+	—
	6 hours	+	—	+	+	—	—	+	—	—	—	—	—
	24 hours	—	—	—	—	—	—	+	—	+	—	+	—
0.5%	20 minutes	+	—	+	—	—	—	+	—	+	—	+	—
	1 hour	+	—	—	—	—	—	+	—	—	—	—	—
	6 hours	—	—	—	—	—	—	+	—	—	—	+	—
	24 hours	—	—	+	—	—	+	—	—	+	—	—	—
0.1%	20 minutes	+	+	+	+	+	—	+	+	+	+	—	—
	1 hour	+	+	+	+	+	—	+	+	+	+	—	+
	6 hours	+	+	+	+	+	+	+	+	+	+	+	+
	24 hours	+	+	+	+	—	+	+	+	+	+	+	+
0.01%	20 minutes	+	+	+	+	+	—	+	+	+	—	+	+
	1 hour	+	+	+	+	+	+	+	+	+	+	+	+
	6 hours	+	+	+	+	+	+	+	+	+	+	+	+
	24 hours	+	+	+	+	+	+	+	+	—	+	+	+

Controls were inoculated with cultures which had been exposed to physiologic salt solution for the same periods of time and otherwise treated in the same way as the medicated cultures. All the control animals exhibited tuberculous lesions, either local or general or both.

time and then treated in the same way. At the end of the exposure, the basket with the garnets was lifted out and washed through three dishes of water and one of physiological salt solution. Then the garnets were transferred into sterile test tubes, each containing 2 c c of sterile physiological sodium chlorid solution, 10 garnets being counted into each tube and 3 or 4 tubes being allowed for each set. These tubes were then shaken thoroughly for one-half hour in a shaking machine to remove the bacteria from the garnets, and the salt solution was then injected subcutaneously into guinea-pigs, the entire amount in one tube being used for one pig. Therefore, there were 3 guinea-pigs in each set, or 60 for each experiment. In this method, the bacteria are in a

thin layer on the garnets so that there is no question of permeation of the layer. There is much less danger of contamination from handling and the guinea-pig response to inoculation is much more delicate than that of the agar tube. Table 2 gives a summary of the results of these experiments.

From this table, it may be seen that 1% was bactericidal in all the compounds except resorcin and even resorcin had in that concentration some bactericidal power, especially if the exposure lasted 24 hours. Creosote and guaiacol had marked bactericidal power in 0.5% concen-

TABLE 2—*Continued*

ANIMAL TEST OF BACTERICIDAL ACTION

Orthocresol						Thymol						Resorcin					
I		II		III		I		II		III		I		II		III	
Local	General	Local	General	Local	General	Local	General	Local	General	Local	General	Local	General	Local	General	Local	General
−	−	−	−	−	−	−	−	−	−	−	−	+	−	+	−	+	−
−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	−	−
−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	−	+	+
−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−
+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
−	−	+	+	+	+	−	−	+	+	−	−	+	+	+	−	+	−
+	+	+	+	+	+	−	−	−	−	−	−	+	+	−		+	+
−	−	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
+	+	+	+	+	+	−	−	−	+	−	−	+	−	+	+	+	+
−	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+	+
+	+	+	+			−	−	−	−	−	−	+	−	+	+	+	+
+	+			+	+	−	−	+	−	−	−	+	+	+	+	+	+
+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
+	+	+	+	+	+	+	+	−	−	+	+	+	+	+	−	+	+
+	+	+	+	+	+			+	+	+	+	+	+	−	−	−	−
+	+	+	+	+	+	−	−	+	+	+	+	+	+	+	+	+	−

tration and thymol killed practically all the organisms in 0.1% dilution. The + sign in the tables indicates that the bacteria developed either in the test tube or in the animal body. If, in table 2, the development of the tuberculous process occurred only in the lymph glands near the point of inoculation, the + sign is under "local." If other glands or other organs in the body are involved, the sign is placed in the column marked "general." It may be noted that results are not entirely consistent even by this method and that the — sign occasionally occurs where we have every reason to expect the + sign, even in the controls. This is probably due to the fact that in the shaking machine some of the tubes may be so placed that the garnets are not shaken violently enough to release a sufficient number of bacteria to cause an infection.

It may in some cases be due to the fact that some of the garnets are not well dried so that the bacteria are washed off before ready for shaking and hence are lost in the germicide or in the wash waters. The results are, however, sufficiently consistent to give us a definite idea of the limit of bactericidal action.

Therapeutic Experiments

Creosote.—Tests on toxicity of creosote were made in order to determine a safe dosage:

One guinea-pig received an intracardiac injection of 3 c c of 1/200 creosote in physiological salt solution or 0.015 c c creosote with no ill effects.

One guinea-pig received an intraperitoneal injection of the same amount, 0.015 c c, with no ill effects.

TABLE 3

Therapeutic Test of Creosote (Set 1)

Duration of Treatment	Total Amount Creosote Received	Local Glands	Liver	Spleen	Lungs	Internal Glands
90 days	Fed 0.462 c c Injected 0.067 ½ c c	—	—	—	—	—
204 days	Fed 1.05 c c Injected 0.067 ½ c c	—	+ ?	—	—	—
293 days	Fed 1.506 c c Injected 0.067 ½ c c	—	—	—	—	—
280 days	Fed 1.44 c c Injected 0.067 c c	+++	+++	—	—	++++
180 days	Fed 0.924 c c Injected 0.067 c c	++	+++	+++	+++	++
112 days	Fed 0.546 c c Injected 0.067 c c	+++	+++	+++	—	+++
141 days	Fed 0.726 c c Injected 0.067 c c	+++	+++	++++	—	+++

One guinea-pig received a subcutaneous injection of 0.1 c c creosote in physiological salt solution with no ill effects and no ulceration of the skin.

Series 1.—These 3 pigs and 6 normal pigs were then inoculated with 0.2 c c dilute emulsion of "old human" tuberculosis.[31] These 9 pigs were fed daily, except Sunday, one pill containing 0.003 c c creosote. Each received in addition one intramuscular injection of 0.005 c c creosote in 1 c c cotton seed oil, one intracardiac injection of 0.01 c c creosote in 2 c c physiological salt solution, 5 subcutaneous injections of 0.005 c c in 1 c c physiological salt solution and 3 subcutaneous injections of 0.0075 c c each in 1 c c physiological salt solution. The injections were made once a week for 10 weeks and the feeding was continued until death. Two of the pigs died immediately after the intracardiac injection, too early to show much general involvement, although the inguinal glands were enlarged and caseous. Table 3 gives results in the other 7 animals.

[31] In all these experiments, the animals of series 1 were inoculated with an amount of culture determined, not by weighing but by the opacity of the suspension. Sufficient sodium chlorid solution was added to make the suspension slightly opalescent. In the animals of series 2, the amount was determined by weight.

Series 2.—In this series, 9 guinea-pigs were inoculated subcutaneously with 0.2 mg. of Corper's 1305[32] in 0.2 c c of physiological salt solution. These were fed daily 0.2 drop creosote in 2 drops cotton seed oil. Five subcutaneous injections, one each week, were given of 2 drops creosote in 1 c c physiological salt solution.

Table 4 gives the results.

TABLE 4

THERAPEUTIC TEST OF CREOSOTE (SET 2)

Duration of Treatment	Total Amount Creosote Received	Local Glands	Liver	Spleen	Lungs	Internal Glands
84 days	Fed 14.22 drops Injected 9.0 drops	+++	+++	+++	+++	+++
105 days	Fed 18.0 drops Injected 9.0 drops	+++	+	+++	+++	+++
123 days	Fed 21.2 drops Injected 9.0 drops	+++	+++	+++	+++	+++
126 days	Fed 21.6 drops Injected 9.0 drops	+++	+++	+++	+++	+++
126 days	Fed 21.6 drops Injected 9.0 drops	+++	+++	+++	+++	++++
128 days	Fed 22.0 drops Injected 9.0 drops	+++	+++	+++	+++	+++
133 days	Fed 22.8 drops Injected 9.0 drops	+++	+++	+++	+++	+
152 days	Fed 26.0 drops Injected 9.0 drops	+++	+++	+++	+++	+++
154 days	Fed 26.4 drops Injected 9.0 drops	+++	+++	+++	+++	+++
220 days	Fed 37.8 drops Injected 9.0 drops	+	+++	+++	+++	+

Four of this set had definite and some fairly large cavities in the lungs—an unusual proportion.

Table 5 gives for comparison the conditions in untreated control guinea-pigs inoculated with the same dose of the same two strains of tubercle bacilli. It may be noted that some of the controls of set 1 failed to develop the disease even after 334 days. In other words, the treatment seems, with creosote, as well as with many other drugs, to lower the resistance of the animal to the disease.

TABLE 5

CONTROLS OF SET 1 OF THIS SERIES

Duration of Treatment	Local Glands	Liver	Spleen	Lungs	Internal Glands
64 days	+	—	—	—	+
96 days	—	—	—	—	—
97 days	+	—	—	—	+
287 days	+	++	+++	+++	+
334 days	—	—	—	—	—
461 days	—	+	++	+	—

Guaiacol Treatment Experiments.—Dec. 15, 1916, to determine the safe dose of guaiacol, 3 guinea-pigs were injected with guaiacol, 1:200 of physiological salt solution. One received 3 c c by intracardiac injection, one 3 c c by intra-

[32] This culture was isolated by Dr. Corper from the sputum of a tuberculous patient at the Chicago Municipal Tuberculosis Sanatorium about 1915, and has been grown in this laboratory ever since; 0.05 mg. injected subcutaneously into guinea-pigs, causes in every case generalized tuberculosis with death in from 4 to 6 months.

peritoneal injection and one received 2 c c by subcutaneous injection. As there were no ill effects except slight infiltration and induration of tissue surrounding the point of subcutaneous injection, the 3 were inoculated with 0.2 c c of a dilute suspension of "old human" tubercle bacilli and 6 other pigs received the same inoculation. Then each of the 9 received daily by mouth a pill containing 0.003 c c guaiacol and also an injection weekly. One injection was intramuscular, one was intracardiac, and seven were subcutaneous. One of these pigs died after 20 days, showing only involvement of a regional lymph gland, while 1 lived 929 days and exhibited at death no tuberculous involvement either local or general. Only 5 of the 9 guinea-pigs in this series showed advanced general tuberculosis; this corresponded fairly well with the controls of the same series in which only 2 of the 6 infected animals showed a general tuberculosis.

Guaiacol Treatment.—Set 2. In this set, Corper's 1305 was used and 0.2 mg. was injected subcutaneously. Ten guinea-pigs were inoculated at the same time. They were fed daily at first 0.2 drop in 2 drops cotton seed oil, then 0.3 drop and later 0.5 drop. Weekly subcutaneous injections of guaiacol were given in physiological salt solution, 1 drop at first, quickly increased to 2 drops in 1 c c of physiological salt solution. Of this set, one died in 21 days, having no tuberculous involvement. All the others exhibited a marked general tuberculosis of lymph glands, liver, spleen and lungs, the degree of involvement being about the same as that shown in the control animals of series 2, summarized in table 6. The last one of the set to die lived 191 days and had received a total of 90 drops of guaiacol.

TABLE 6

Controls for Set 2 of all these Experiments

Duration of Disease	Local Glands	Liver	Spleen	Lungs	Internal Glands
84 days	+++	++	++	+	—
98 days	+++	+++	+++	+++	+++
107 days	++	+	+	+	++
107 days	+++	+++	+++	+++	+++
114 days	+++	+++	+++	+++	+++
122 days	+++	++++	++++	+++	+++
138 days	+++	++++	++++	++++	+++
159 days	+++	+++	++++	++++	+++
163 days	+++	++++	+++	+++	+++
223 days	+++	++++	+++	+++	+++

Two of the animals had lungs containing cavitiees.

Creosol Treatment Experiments.—The first set consisted of 9 guinea-pigs, 3 of which had been previously used to determine the safe dose of creosol, and all were inoculated in January, 1917, with 0.2 c c of dilute emulsion of "old human." Four died or were killed too early to show any development of the disease. Two of these died immediately after an intracardiac injection, one from pneumonia and one from being crushed in the cage. In 2 of these early deaths, in which the guinea-pigs had lived 2 weeks, the animals showed early signs of tuberculosis, such as enlarged glands, and one had a few young tubercles in the lungs. The others were fed daily 0.2, 0.3 and 0.5 drop of creosol in cotton seed oil up to the time of death, and received 11 subcutaneous injections of creosol in physi-

ological salt solution at weekly intervals. Four of the 5 remaining animals in this set showed generalized tuberculosis, a much larger proportion than was seen in the untreated controls of the same set.

Creosol Treatment.—Set 2 was inoculated Feb. 4, 1918, with 0.2 mg. of culture 1305 and fed daily 0.2 of a drop for 12 days, 0.3 of a drop, then the rest of life 0.5 of a drop in cotton seed oily daily, except Sundays. The animals were injected weekly for 6 weeks with 2 drops in physiological salt solution. Ten guinea-pigs were used in this series. The first to die lived 80 days and the last 212 days; all showed a marked generalized tuberculosis, corresponding to the untreated controls of the same series (see table 6).

Thiocol Treatment.—Series 1: Jan. 3, 1917, six pigs were inoculated subcutaneously with 0.2 c c weak suspension of "old human" culture. From Jan. 4 on, they were fed daily one 5 mg. pill. After Jan. 18, they were fed two 5 mg. thiocol pills daily. On Jan. 10 and each week thereafter they were injected subcutaneously 5 mg. in 1 c c physiological salt solution the first 3 weeks and afterward 10 mg. in 1 c c physiological salt solution. The thiocol was easily soluble and seemed to have no ill effects. Four of the 6 animals of this set showed marked generalized tuberculosis.

Thiocol Treatment.—Series 2: Ten guinea-pigs were inoculated Feb. 4, 1918, subcutaneously with 0.2 mg. of Corper's 1305. From Feb. 5 to March 14, they were fed daily 5 mg. of thiocol and after that, 10 mg. Once a week, they were injected subcutaneously, 5 mg each time the first two weeks and 10 mg. each time thereafter. One of the pigs in this set died in 50 days; the last one died after 287 days, having received 2350 mg. of thiocol. All showed marked generalized tuberculosis, and no effect from the treatment.

Styracol Treatment.—Set 1: Jan. 3, 1917, the guinea-pigs were inoculated with 0.2 c c of "old human." Styracol is insoluble in water and the injections were therefore made in cotton seed oil, some intramuscular and some subcutaneous. These injections were made once a week and pills were fed daily except Sunday, 5 mg. for the first 2 weeks and 10 mg. from then on. The only effect of this treatment seen was an increase of generalized tuberculosis over the controls, since all 6 treated animals developed marked generalized tuberculosis while only 2 of the 6 controls showed any involvement more than a slight enlargement of the regional lymph glands.

Styracol.—Set 2: Ten guinea-pigs were inoculated with 0.2 mg. 1305. Treatment was the same as in set 1. One of the 10 guinea-pigs of this set died in 42 days with no tuberculous involvement except in the regional lymph glands, all the other pigs of this set, the last of which lived 247 days and received 1950 mg. of styracol, showed marked generalized tuberculosis, corresponding to controls given in table 6.

Orthocresol, Metacresol, Paracresol and Thymol.—Treatment: The guinea-pigs received inoculations with 0.2 mg. of culture 1305. They were fed daily 0.001 c c of the indicated drug in pill form. After the first 3 weeks, they were fed 0.002 c c daily. They also received subcutaneous injections once a week, usually 0.003 c c in water, but part of the time 0.006 c c was injected. The weekly injections and daily feedings were kept up until death. Thymol, being a solid, was weighed and 1 or 2 mg. were fed daily and 3 or 6 mg. were injected. Of the animals treated with these four drugs, none showed any effect of treatment, if we except a slight diminution of tuberculous involvement in pigs treated with paracresol and thymol. The first of the paracresol set to die lived 120 days and showed practically no generalized tuberculosis, while

even one dying after 134 days showed only a few small tubercles in the lungs, liver and spleen. The ones dying later, however, had advanced involvement of all the internal organs which are subject to tuberculous infection. Of the thymol set, 1 died in 58 days, 1 in 80 days and a third after 115 days with no or slight general involvement and 1 animal is still living and apparently well after 239 days. However, 1 dying after 136 days and 1 after 147 days showed marked generalized tuberculosis. The controls and the animals treated with orthocresol and metacresol showed without exception advanced tuberculosis, even in those dying at 73 and 87 days. The longest period of life of the controls was 171 days, of the metacresol treated, 107 days and of the orthocresol treated, 188 days.

All of the compounds of this series have shown so little local toxicity that it was at no time necessary to stop the injections on account of infiltrations, necrosis and ulcerations of the skin, and so little general toxicity that neither the weights, which were taken weekly as a guide to treatment, nor the general condition of the animals ever suggested the desirability of stopping either the feeding or the injections. Twelve normal guinea-pigs have been treated for 125 days with thymol and the cresols, using 3 animals for each drug. The same doses and method of administration have been used with these as with the tuberculous guinea-pigs. Two of these nontuberculous pigs died early from acute cage infections, but the rest are living, in good condition and gaining weight. Hence we may say that all of these drugs are relatively innocuous to guinea-pigs. We have not used intravenous injections in our experiments, since frequent intravenous injections into the same guinea-pig are difficult to make and the intracardiac injections are dangerous.

TABLE 7

Amount of Organ Involvement in Treated Tuberculous Guinea-Pigs

Control	Creosote	Guaiacol	Creosol	Thiocol	Styracol	Orthocresol	Metacresol	Paracresol	Thymol
40	55 5/7	66 1/9	68	66⅔	85				
97	98.5	88	94	89.3	87				
98⅓	.....	.....	.....	.....	.....	94	91⅔	78.6	62

In estimating the therapeutic effect of drugs, they must be judged in several ways. In human patients, it is usual to say that a drug checks or lessens cough, increases or diminishes expectoration, lessens pain and relieves other symptoms. Since experimental animals exhibit few, if any, of these symptoms, we cannot judge in this way. At the death of the animal, we can determine whether the disease is present or absent in the organs, and, if present, how its degree compares with that in control animals which died at approximately the same time after inoculation. We can also compare the duration of the disease in the treated animals with that in the control animals. In order to compare averages of these drugs with reference to the extent of the disease after their therapeutic use, we have endeavored in table 7 to represent the degree of the disease in terms of percentage, calling an extreme

involvement of all the organs 100% and so on down to 0 where all were — and then averaging these percentages.

It may be seen from table 7 that in the first set, which represented the less virulent strain of tubercle bacilli, the degree of the disease was much greater in the treated animals than in the controls, suggesting a possible stimulation of the growth or reduction in resistance. In the second set, the degree of the disease averaged less in the treated animals than in the controls, except in the case of creosote, while in the third set, the degree of the disease was much less in the treated animals than in the controls. A part of this difference may be due to earlier death of the treated animals, since the duration of life may influence the extent of the disease.

The prolongation of life, if at all marked and consistent, may also indicate some beneficial influence of a drug. For these reasons, it seems best, for purposes of comparison, to insert table 8 giving the average duration of life.

TABLE 8

DAYS OF DURATION OF LIFE OF TREATED TUBERCULOUS GUINEA-PIGS

Control	Creosote	Guaiacol	Creosol	Thiocol	Styracol	Orthocresol	Metacresol	Paracresol	Thymol
223.2	185 4/7	285.0	134.2	146.0	150.3				
131.5	135.1	115.3	139.0	147.6	123.9				
157.5	.....	.....	.....	.....	.....	114.6	86.5	140	107.2

From all these facts, we must conclude that none of the compounds belonging to the guaiacol series, so far as we have tested them, has shown definite therapeutic action in experimental tuberculosis in guinea-pigs.

SUMMARY

Despite the extensive use of creosote and related compounds in the treatment of tuberculosis, practically no evidence exists as to the susceptibility of B. tuberculosis to antiseptics of this class, or as to their influence on the course of tuberculosis in experimental animals. A study of these problems showed that:

Virulent human tubercle bacilli are inhibited from growth (bacteriostatic action) on artificial mediums containing a concentration of 0.01% or 1 part in 10,000, each of resorcin, thymol, paracresol, orthocresol and metacresol; 0.05% (1:2,000) is the lowest concentration which completely inhibited in the case of creosol and pyrocatechin. Guaiacol, creosote, hydroquinone and guaiacol cacodylate required a concentration of 0.1% (1:1,000) to inhibit growth completely.

Sodium guaiacolate inhibited completely at 1.7%, and partially at 0.8%. Thiocol did not inhibit in 1% concentration and styracol, which is insoluble, did not inhibit in 10% concentration (suspension).

Bactericidal tests, in which the capacity to grow on agar after exposure of clumps of tubercle bacilli to the antiseptic was the measure of action, showed that the bactericidal power of these substances is low. Exposure to even 1% solutions of pyrocatechin, hydroquinone, resorcin, and 0.5% solution of creosol, for periods from 10 minutes to 48 hours, entirely fails to kill human tubercle bacilli. Metacresol and paracresol kill in 1% concentrations after exposure for one hour, but not after 10 minutes, while orthocresol reduces growth after 1 hour, and kills in 6 hours. Thymol kills even in 10 minutes at 1% concentration, but 0.1% concentration does not kill even in 48 hours. Weaker concentrations of all these antiseptics were, of course, without bactericidal effect.

A bactericidal test was made with the tubercle bacilli exposed to the antiseptics when in a thin layer on the surface of garnets, and viability determined by inoculating guinea-pigs with the treated bacilli washed from the garnets. Resorcin in 1% solution killed the tubercle bacilli only after 24 hours' exposure. Orthocresol killed in 1% concentration even in 20 minutes, but 0.5% did not kill even in 24 hours. Creosote and guaiacol both killed most of the bacilli in 0.5% concentration, even in 20 minute exposure, but 0.1% concentration was not bactericidal in 24 hours. Thymol was bactericidal in 0.1% concentration, even in an exposure of 20 minutes, but 0.01% was not bactericidal in an exposure of 24 hours.

Therapeutic tests were made on guinea-pigs injected subcutaneously with two strains of human tubercle bacilli, one highly virulent and the other much less so. The animals were then given several doses of the drug by the intracardiac, intramuscular and subcutaneous routes, and daily feedings of pills containing the drugs, the following being tested: creosote, guaiacol, creosol, thiocol, styracol, orthtocresol, metacresol, paracresol and thymol. In all, 106 guinea-pigs were thus treated for long enough periods to observe the results (besides the control experiments). Apparently the animals injected with the less virulent tubercle bacilli showed more active tuberculosis than the controls, as if the treatment had lowered their resistance or stimulated the bacilli. With the more virulent bacilli the extent of the disease was perhaps slightly less in the treated animals, probably because they commonly died a little sooner than the controls.

Our experiments show that substances of the creosote series do not possess a high bactericidal power for the tubercle bacillus in vitro, and apparently not in vivo. This result is not surprising in view of the observations of DeWitt and Sherman [30] that tubercle bacilli are rather less susceptible to fat-soluble, and more susceptible to water-soluble antiseptics, than bacteria less rich in fat than the tubercle bacillus. Also by their observation that fat-soluble dyes do not readily penetrate tubercle bacilli, while certain fat-insoluble dyes (e. g., methylene blue) stain them well. Apparently the lipin-rich character of the tubercle bacilli does not make them vulnerable to fat-soluble antiseptics, but rather the reverse.

The figures given above for the bactericidal power may be compared with those obtained by DeWitt and Sherman, using similar methods. Phenol kills in 1% concentration, and shows some effect in 0.1% concentration. Formaldehyd kills in 1% in 1 hour, in 0.1% in 24 hours. Ethyl alcohol, 25%, kills in 1 hour or less. Acetone, chloroform and ether have little or no tuberculocidal action; toluene and iodin show slight influence. Mercuric chlorid kills in 0.001% in 24 hours, 0.1% in 1 hour; gold chlorid in 0.005% kills in 24 hours, as do 0.25% silver nitrate, 0.1% gold tricyanid and 5% copper chlorid. Evidently creosote. guaiacol and the cresols, have about the same tuberculocidal power as phenol, which is distinctly not high. The dihydroxy phenols, resorcin, hydroquinone and pyrocatechin, seem to be less active than the monhydroxy phenol. Thymol was, in all experiments, distinctly, although only slightly, more bactericidal than the other substances tested. This agrees with the statement of Bechhold and Ehrlich [9] that addition of alkyl groups to phenols increases their disinfectant action.

The failure to observe any beneficial therapeutic effect on tuberculous guinea-pigs is, in view of the low bactericidal power of the substances tested, to be expected. It does not mean, however, that these substances may not have value in open tuberculous infections in man in which other bacteria than B. tuberculosis are involved. But it does substantiate the opinion that seems to have been generally reached by careful clinical observers, that creosote and guaiacol do not have a specific action on tuberculous infection.

[30] Jour. Infec. Dis., 1914, 15, p. 245.